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This project focuses on the role of nuclear receptor coactivators in steroid dependent regulation of cell growth in pathogenesis of breast cancer . A novel nuclear receptor coactivator of transcription, p300 and CBP associated factor (p/CAF) has been shown to be required for estrogen-, thyroid hormone-, and retinoic acid-dependent gene expression. The histone acetyltransferase activity of p/CAF was demonstrated to play essential role in nuclear receptor dependent gene expression and it appeared to be transcription factor specific. The ligand-dependent association of p/CAF with nuclear receptor depends upon nuclear receptor corepressor (NCoR) dismissal. These data suggest that p/CAF complex plays a central role in nuclear receptor dependent gene regulation, a key element in steroid dependent cancer development.

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TABLE OF CONTENTS:

Introduction:

In a large number of breast cancer tumor, proliferation of cells is stimulated by estrogen and can be further enhanced through thyroid hormone. In contrast, retinoic acid exerts an inhibitory effects on proliferation of these cells, as it does on cells in hematopoetic system. However, the precise molecular mechanisms through which these hormones exert their effects and regulate cell growth remain obscure. Nuclear receptors exhibit ligand-dependent interactions with a series of coactivators that include CBP/p300 (1, 2, 3) and the biochemically-identified p160 proteins (4) which have been identified as the structurally-related SRC-1/NCoA-1 (2, 5), TIF2/GRIP-1/NCoA-2 (6) and p/CIP(7, 8).

The C/H3-E1A interaction domain of CBP/p300 interacts with the p300/CBP-associated factor (p/CAF)(9, 10), which is highly related to the yeast transcriptional adaptor GCN5, a histone acetyltransferase (11). Acetylation of lysines localized at histone N-terminal tail neutralizes positive charges leading to destabilization of chromatin compact structure and subsequently increases accessibility of the transcritional machinery to a promoter(12, 13). Thus the presence of histone acetyltransferase activity in the coactivators of transcription explains, at least in part, the function of these proteins in the regulation of gene expression (12, 13, 14). Both CBP/p300 and p/CAF exhibit potent histone acetyltransferase activities (9, 15) as well as against other non-histone, physiological substrates (16). Also the p160 factors are reported to possess C-terminal HAT activity (8, 17). Moreover histone acetyltransferases has been recently implicated in pathogenesis of cancer. In acute myeloid leukemia, the translocation t(8;16)(p11;p13) fuses the MOZ zinc finger motifs and putative acetyltransferase domain with a largely intact CBP causing possibly aberrant chromatin acetylation (18). The amplification of AIB1 gene, a human homolog of p/CIP has been reported in estrogen-positive breast and ovarian cell line as well as in specimens of primary breast cancer (19). High expression of AIB1 which can bind directly to estrogen receptor in ligand-dependent manner, enhance estrogen-dependent transcription and has histone acetyltransferase activity may contribute to development of steroid dependent cancers (19, 20).

This project focuses on the role of nuclear receptor coactivators in the regulation of gene expression in pathogenesis of breast cancer. During the course of this study (first year), novel nuclear receptor coactivator, p/CAF has been characterized and its role in activation of transcription was examined (10). It has been demonstated that p/CAF plays an essential role in nuclear receptor regulated gene expression.

Materials and Methods

Generation of Anti-p/CAF IgG

A cDNA fragment corresponding to p/CAF amino acids 466-832 generated by restriction digestion from AC611 cDNA clone was subcloned into the pQE31 vector (Quiagen) containing an in-frame His tag and recombinant HIS-tagged proteins were generated and purified by nickel chelate chromatography to homogeneity. The purified recombinant proteins were injected into guinea pigs , and anti-bodies were generated and affinity purified on Protein A-Sepharose FPLC using standard procedures(21).

Isolation of p/CAF gene

The screen for nuclear receptor coactivator with histone acetyltransferase activity was carried out employing Gene Trapper cDNA Positive Selection System (Life Technologies. Inc.). The undoubtfull advantage of this particular screening technology is a very fast data collection process but it requires to use a plasmid cDNA library. In this system the biotinylated oligonucleotide complementary to a segment of target cDNA is hybridized to ssDNA, a product of conversion of ds phagemid DNA containing cDNA library using Gene II endonuclase and E. coli Exonuclease III. Hybrids are captured on streptavidine-coated paramagnetic beads and retrieved by magnet from solution. After release, the desired cDNA clone is recovered by specifically prime conversion to ds DNA following by transformation. A cDNA clone, AC611 (2 Kb) was isolated from a human fetal cDNA library (Gibco BRL) using oligonucleotide: TTG GAT ACT TTA AGA AAC AGG G corresponding to amino acids 218-225 of yGCN5 protein. The oligonucleotide sequence was based on the sequence found in randomly sequenced human complementary DNAs (Database of Gene Bank EST Division), clone # N39522 which

revealed significant homology to yeast GCN5 protein. Obtained clone turned out to be a partial clone of the recently published p300 and CBP associated factor (p/CAF) corresponding to amino acids 301-832 of full length p/CAF(9). Subsequently, a full length p/CAF cDNA clone, pCX-Flag-p/CAF, was kindly provided by Y. Nakatani (9).

Yeast Two-hybrid Interaction Assays

The yeast strain EGY 48, the LexA-b galactosidase reporter construct (PSH 18-34) and the B42 parental vectors (PEG 202 and PJG 4-5) were all previously described(22). Various p/CAF fragments obtained by PCR or restriction digestion were subcloned into PEG 202 bait vector. DNA fragments of p/CIP , SRC-1, CBP or RAR proteins were generated either by using an appropriate restriction digest or by PCR and subcloned into PJG 4-5 prey vectors. EGY 48 cells were transformed with the *lac Z* reporter plasmid pSH 18-34 with the appropriate bait and prey vectors, and plated out on -Ura-His-Trp medium containing 2% galactose. Isolated yeast colonies were then allowed to grow in the same liquid medium, followed by assaying for b galactosidase, as previously described(23).

Transient Transfections and Reporter Assays

Transfection experiments were conducted in either HeLa cells using the standard calcium phosphate procedure(23). Cells were co-transfected with 1.0ug of a (UAS)3-luciferase reporter and the 5ug of GAL4 fusion proteins, and harvested 48 hrs later in order to perform luciferase activity assay.

Immunoprecipitations, Western Blot, GST-TNT Interaction Assays

Whole cell extracts were prepared by lysing the cells in NET-N buffer containing 50 mM Tris (pH 7.6), 5 mM EDTA, 0.3 M NaCl, 1mM DTT, 0.1% NP-40 and protease inhibitors (.2 mM PMSF, 10 ug/ml each of leupeptin, pepstatin and aprotinin), centrifuged at 30K for 1 hr at 4°C and the supernatant was stored at -80° C until use. For co-immunoprecipitation assays, 1 mg of cell extract was incubated in the presence of 2 ug of anti-p/CIP, or anti-Flag, or anti-retinoic acid receptor (RAR) IgG for 2 hrs at 4° C. The immune complexes were then precipitated with Protein-A Sepharose. Protein complexes were resolved by SDS-PAGE(24) and

Western blotted and probed using 1 ug/ml of an anti-HA, or anti-Flag, or anti-p/CIP. TTNPB and LG629 were kindly provided by R. Heyman.

GST-RAR and GST-CBP (1458-1891) were generated as described(2). 25 ul of GST-sepharose beads containing 3-6 ug of the GST recombinant proteins were incubated with $5x10^5$ CPM of ^{35}S-labelled p/CAF proteins generated by *in vitro* transcription and translation for 2 hrs at 4° C. The complexes were washed 5 times with NET-N buffer, resolved by SDS-PAGE and fluorographed.

Mutagenesis

Mutation in p/CAF were introduced by site-directed mutagenesis using the quick change mutagenesis kit according to the manufacturers instructions (Stratagene). Double stranded oligonucleotides were designed such that the wild type sequence corresponding to amino acids Y615/F616 (acetyl CoA binding site) in p/CAF cDNA were substituted with alanines in order to generate mutant of p/CAF lacking HAT activity pCMV-p/CAF(HAT_m). A similar strategy was used to obtained mutants of CBP. CBP was expressed in Baculovirus and tested for histone acetyltransferase activity in solution using histones as a substrates (25).

Single Cell Microinjection Assay

Insulin-responsive Rat-1 fibroblasts were seeded on acid washed glass coverslips at subconfluent density and grown in MNE/F12 medium supplemented with 10% fetal bovine serum, gentamicin and methotrexate. Prior to the injection, the cells were rendered quiescent by incubation in serum-free medium for 24-36 hours. Plasmids were injected into the nuclei of cells at a final concentration of 100 mg/ml. Peptides were injected at a concentration of 200 mM. Either preimmune IgG of the appropriate species or antibodies directed against p/CAF p/CIP, NCoA-1, or CBP were co-injected and allowed the unambiguous identification of the injected cells. Microinjections were carried out using an Eppendorf semiautomated microinjection system mounted on an inverted Zeiss microscope. Approximately one hour after injection, the cells were stimulated, where indicated, with the appropriate ligand. In the case of rescue experiments, the cells were stimulated with ligand six hours after injection, to allow protein expression. After overnight incubation, the cells were fixed and then stained to detect injected IgG and b-

galactosidase expression(2, 26). Injected cells were identified by staining with tetramethylrhodamine-conjugated donkey anti-rabbit IgG.

Results and Discussion:

The recent finding that some of the coactivators have a intrinsic histone acetyltransferase activity promted a screen for the nuclear proteins that possess homology to HAT domain found in yeast coactivators and are required in nuclear receptor dependent gene activation. HAT activity in coactivator complex is thought to play essential role during activation of transcription by regulating acetylation of nearby histones. Histone acetyltransferase, GCN5 was one of several proteins identified by genetic selection in, including ADA2 (27) that were required for function of certain classes of activation domains (11). This promted a screen for coactivators involved in nuclear receptor gene activation containing a histone acetyltransferase domain homologous to yeast GCN5 (Statment of Work; Task 1). This screen was performed using Gene Trapper cDNA Positive Selection System (Life Technologies. Inc.) (Methods). Sequence analysis of isolated clone revealed that it was a partial clone of the recently published p300 and CBP associated factor (p/CAF) corresponding to amino acids 301-832 of full length p/CAF(9) (Statment of Work; Task 2).

In order to define the potential function of p/CAF in nuclear receptor-dependent regulation of gene transcription a single cell microinjection of specific blocking IgG against p/CAF (aa 465-832) (Methods) developed in guinea pig (Statment of Work; Task 4, 5) revealed that p/CAF was required for ligand-dependent activation, not only of estrogen-, but also of thyroid hormone- and retinoic acid-dependent transcription units (Fig. 1). Other transcription units, including a promoter under the control of multiple SP1 sites, were unaffected by anti-p/CAF IgG (Fig. 1), suggesting that p/CAF is not required for a function universal to all transcription units (Statment of Work; Task 5). In cases where no specific IgGs were used, preimmune rabbit or guinea pig IgG was coinjected, allowing the unambiguous identification of

the injected cells in addition to serving as a preimmune control for the experiment (2, 7). The observed specificity of p/CAF function is consistent with observations concerning selective functions of GCN5 in yeast (11).

I am in the process of gathering data on the pattern of expression of p/CAF during embryogenesis as well as in adult tissues using *in situ* hybridization and western blotting analysis (Statment of Work; Task 3) though preliminary data suggest ubiguitous expression pattern (the full report of p/CAF expression pattern will be presented after it is completed).

Coimmunoprecipitation assays performed from cell extracts revealed that p/CAF exhibits ligand-dependent recruitment to the retinoic acid receptor coactivator complex, and p/CAF binding was abolished by binding of an RAR antagonist (Fig. 2A). However, there was only minimal ligand-dependence of this interaction in a yeast two-hybrid assay, with the interaction domain mapping to the N-terminus (aa1-351) of p/CAF (Fig. 2B). Using the avidin, biotin complex DNA assay to assess protein interactions on DNA bound receptors, there was no detectable ligand-dependence for association of p/CAF with RAR/RXR heterodimers (Fig. 3A, B). However, this interaction between nuclear receptor and p/CAF was inhibited by the nuclear receptor corepressor (NCoR) **(28)** and restored on ligand-induced dismissal of NCoR from the receptor (Fig. 1C) **(29)**, suggesting that ligand-dependent association of p/CAF in cells depends upon release of the nuclear receptor corepressor complex(**30**). In contrast to the p160 coactivators, p/CAF interactions occurred independent of the AF2 domain (Fig. 3B). Together, these data reveal that the ligand-dependent recruitment of p/CAF to the activated nuclear receptor is distinct from the AF2-dependent mechanism of interaction between SRC-1/p/CIP and nuclear receptors **(4, 31)**.

GST pull-down and yeast two hybrid assays revealed that, in addition to the previously described interaction between p/CAF and the C/H3-E1A interaction domain of CBP (9), the N-terminal region of p/CAF was also capable of direct interaction with the N-terminal region of CBP (Fig. 4) (Statment of Work; Task 6, 8). Consistent with the observation that both NCoA-1/SRC-1 (2, 5, 17) and p/CIP (7) could be coimmunoprecipitated with p/CAF present in cell extracts (Fig. 5B and (8, 17)), yeast two hybrid assays (Fig. 2C) demonstrated that the N-

terminus of p/CAF mediated interactions with SRC-1 (Fig 5A), while the most effective p/CIP interaction domain with p/CAF was delineated to aa 649-725 (Fig. 5C, D), corresponding to the conserved regions of yeast and human GCN5 that interact with ADA2 (11). These findings revealed the presence of multiple potential interaction interfaces between members of the coactivator complex.

Based on a number of mutagenesis studies demonstrating that single amino acid substitutions, particularly in acetyl-CoA binding region of acetyltransferases, resulted in loss of enzymatic activity (32), a p/CAF mutant protein harboring a substitution of two conserved residues (Y616/F617 to A616/A617) was generated. This mutant had no intrinsic HAT activity (p/CAF_{HAT^-}) as was shown in liquid histone acetyltransferase assay (Fig. 6A). Two amino acid mutation (L1690/C169 to K1690/L1691) in CBP abolished its histone acetyltransferase activity (CBP_{HAT^-}). The CBP_{HAT^-} mutant was provided from Dr. C. Glass laboratory.

These mutants of p/CAF and CBP lacking a histone acetyltransferase activity allowed for an evaluation of the role of the histone acetyltransferase activity of p/CAF and CBP in transactivation function of specific classes of transcription factor. Blockade of both CBP and p/CAF activity by coinjection of both specific blocking IgGs, which almost abolished the transcriptional activity of retinoic acid receptor or CREB, was entirely reversed by coinjection of expression vectors expressing wild-type CBP and p/CAF (Fig. 3B). Conversely, expression of both factors, mutated to abolish acetyltransferase function mutation (HAT^-), failed to effectively rescue activation (Fig. 6B). A failure to rescue retinoic acid receptor activity was also observed with expression of wild-type CBP and p/CAF_{HAT^-} (Fig. 3B). However, expression of wild-type p/CAF in the presence of CBP_{HAT^-} fully restored the ligand-dependent activation function of the retinoic acid receptor (Fig. 3B). In contrast, for CREB function, the HAT activity of CBP was required, while that of p/CAF was of minimal importance (Fig. 6B). These transcription units served as internal controls, because both HAT^- coactivators were functional, with differences reflecting the distinct requirements for specific HAT activity by different classes of transcription factors. In the near future these point mutants of p/CAF and CBP lacking histone

acetyltransferase activity will be evaluated as a potential dominant negative mutants in estrogen dependent gene expression in MCF-7 cell line (Statment of Work; Task 9).

In order to evaluate a function of different interaction domains of p/CAF, microinjection assays were performed, using retinoic acid receptor- or CREB- dependent promoter, to assess the ability of N- and C-terminally truncated p/CAF proteins to function in retinoic acid- and cAMP-dependent transcription (Statment of Work; Task 10). Deletion of the N-terminus of p/CAF (aa 1-518) did not significantly impair its function on either retinoic acid- or cAMP-stimulated transcription; however, the C-terminus was required for function of both transcription factors (Fig. 7). These data suggest that alternative interaction interfaces can be utilized in recruitment of specific, required factors into the coactivator complex. P/CAF activity is lost with deletion of the p/CIP interaction domain (Fig. 7), consistent with the functional effects of ADA2 domain in GCN5 (11).

While nuclear receptors required p/CAF, p/CIP, SRC-1, and CBP, the protein kinase A-dependent activation of CREB(1) required CBP, p/CAF and p/CIP, but not SRC-1 (Fig. 8). Because the C'-terminal domain of p/CAF that selectively associates with p/CIP is distinct from the SRC-1 interaction domain (Fig. 5), p/CAF could potentially provide a molecular platform for differential positioning of components of the p/CAF/p/CIP/CBP/p300/SRC-1 complex in a promoter-specific manner. While STAT-1 is associated with, and requires the action of, both CBP/p300 (33) and p/CIP, STAT-1 proved not to require either p/CAF or SRC-1, as blocking IgGs against these factors (7) each failed to inhibit activity of the interferon-g responsive element (Fig. 8). Thus different classes of transcription factors - nuclear receptors, CREB and STAT - appear to functionally require distinct components of the coactivator complex, including CBP/p300, SRC-1, p/CIP, and p/CAF. These data are most compatible with a model in which there are multiple possible configurations of the specific components of the coactivator complex recruited by different transcription factors.

Conclusions:

These data suggest a selectivity in the specific histone acetyltransferase activity required for function of distinct classes of transcription factors. The histone acetyltransferase activity of p/CAF, but not of CBP, appears to be indispensable for nuclear receptor activation, where CBP is likely to be recruited based on interaction with a complex containing p/CAF and SRC-1. Conversely, the HAT activity of CBP, directly recruited by phosphorylated CREB(1) is required for its transcriptional function. These observations suggest that transcription factor-specific differences in configuration and content of the coactivator complex dictate requirements for specific acetyltransferase activities, providing an explanation, at least in part, for the presence of multiple HAT components of the complex. In the case of nuclear receptors, p/CAF and SRC-1/p/CIP bind receptors in a ligand-independent and ligand-dependent fashion, respectively, with p/CAF recruitment apparently dependent upon dismissal of the N-CoR, corepressor complex (Fig. 3). More recently p/CAF has been found in the complex with more than 20 distinct subunits (34) homologous or identical to components of TFIID complex suggesting that p/CAF complex might play an alternative role to TFIID complex in transcription (34, 35). Together, these data strongly suggest that p/CAF plays a central role in nuclear receptor-dependent gene expression. Moreover, considering the recent observations that overexpression of histone acetyltransferase targeted to chromatin may contribute to cancer development dysregulation of p/CAF function might contribute to steroid dependent cancer such as breast cancer.

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FIGURE LEGENDS

Figure 1. P/CAF is a nuclear receptor coactivator. Microinjection of purified anti-p/CAF IgG blocked retinoic acid (RA)-, thyroid hormone (TR)- or estrogen receptor (ER)-dependent activation of promoters containing corresponding response elements in Rat-1 cells, but not SP1-driven reporter expression. Similar results were obtained in three independent experiments. All results are representative of experiments performed in triplicate, in which more than one thousand cells were injected for each experimental condition.

Figure 2. P/CAF interacts directly with nuclear receptor. (**A**) Immunoprecipitation with anti-RAR antibodies of nuclear extracts from HeLa cells transfected with Flag-tagged p/CAF, and treated with all-trans-RA (10^{-7}M) or an antagonist, LG629 (10^{-7}M) reveals ligand-dependent co-immunoprecipitation of p/CAF and RAR, detected using monoclonal anti-Flag IgG. (**B**) A yeast two hybrid assay recorded specific interaction between indicated p/CAF fragments (baits) and the C-terminal domain of retinoic acid receptor (prey) in the presence or absence of retinoic acid. Duplicates differed by less than 10%.

Figure 3. Ligand-dependent association of p/CAF with a nuclear receptor coactivator in cells depends upon release of the nuclear receptor corepressor complex. Interaction between p/CAF and RAR was tested in previously described DNA-dependent assay (avidin, biotin complex DNA) for protein-protein binding. Bacterially-expressed RAR, or RARDAF2, and RXR were bound to biotinylated oligonucletides corresponding to a DR5 (RARE), captured on streptavidin-agarose and incubated with [^{35}S]-labelled p/CAF in the presence of pan-agonist 9-cis-RA (10^{-6}M) or antagonist, LG629 (10^{-6}M), and analyzed by SDS-PAGE electrophoresis. Bacterially expressed NCoR (1mg) (28) was incubated with receptor-DNA complexes before p/CAF addition.

Figure 4. Mapping of interaction domains between p/CAF and CBP. (**A**) A yeast two hybrid interaction assay using p/CAF(1-654) (bait) and CBP fragments (preys), revealing two

independent interaction domains: (aa 1-450 and 1068-1891) of CBP. (**B**) Both CBP interaction domains interacts with N-terminal domain of p/CAF in yeast two hybrid assay but not with C-terminal domain of p/CAF. (**C**) GST pull-down assays with the CBP C-terminal interaction domain and fragments of [^{35}S]-labelled p/CAF. 5 ug of GST-CBP(aa 1069-1891) protein was incubated with various [^{35}S]-labelled p/CAF derived fragments, and specifically bound fragments detected as above; and (**D**) GST pull-down of [^{35}S]-labelled p/CAF (1-351) with N- and C-terminal GST-CBP fragments.

Figure 5. P/CAF interacts with nuclar receptor coactivators SRC-1 and p/CIP. Yeast two hybrid interaction between (**A**) p/CIP (aa 947-1084) (prey) or (**B**) SRC-1 (aa 896-1200) (prey) and fragments of p/CAF (baits) revealed a selective C-terminal p/CIP interaction domain and N-terminal SRC-1 interaction domains. Duplicate determinations differed by <10%. (**C**) Immunoprecipitations of HeLa extracts from cells cotransfected with Flag-tagged p/CAF and HA-tagged NCoA-1/SRC-1 expression vectors were subjected to Western blot analysis employing anti-HA for detection. (**D**) Mapping of interactions between p/CAF, nuclear receptor, coactivators, and CBP. Schematic of CBP, p/CAF, SRC-1 and p/CIP, and their interaction domains. NR ID, nuclear receptor interaction domain; CBP ID, CBP interaction domain.

Figure 6. Histone acetyltransferase activity of p/CAF is required for RARE-dependent gene activation. (**A**) Generation of mutations in the p/CAF (Y616/F61 to A616/A617) (p/CAF$_{HAT^-}$) that abolish detectable acetylation of histones using [^{14}C]acetyl-CoA as substrate. Activity was determined by liquid histone acetyltransferase assay using bacterially-expressed p/CAF. (**B**) Requirement for p/CAF and CBP histone acetyltransferase activity in retinoic acid receptor and CREB function tested in single cell microinjection assay. In these experiments, specific IgGs against CBP and p/CAF were coinjected with vectors directing expression of wild type wild-type or HAT$^-$ mutant of p/CAF and CBP. The ability of retinoic acid (10^{-7}M) or forskolin (10^{-6}) to activate the appropriate reporter gene was then determined. Similar results were obtained in three independent experiments of similar design.

Figure 7. Role of HAT and other domains in functions of the coactivator complex on RARE- and CREB-dependent gene activation. The ability of wild type p/CAF, p/CAF(ΔN) (aa518-832), p/CAF(ΔC) (aa1-654), p/CAF(Δ654-682) or p/CAF(ΔBr) (D745-832) to rescue retinoic acid-dependent activation of a RARE/LacZ reporter (**left**) or forskolin-dependent activation of the CREB-dependent reporter (**right**) in single cell microinjection assays; similar results were obtained in three separate experiments.

Figure 8. Transcription factor specificity in required coactivator complex components. (**Left**) Inhibition of retinoic acid receptor activity by microinjection of anti-p/CAF, -p/CIP or -NCoA-1/SRC-1 or -CBP IgG; (**midle**) Inhibition of CREB activation by anti-CBP, anti-p/CIP or anti-p/CAF IgG, but not by anti-SRC-1 IgG; (**right**) Inhibition of Interferong-dependent activation of the GAS-dependent promoter by anti-CBP or anti-p/CIP IgG but not by anti-p/CAF and anti-SRC-1.

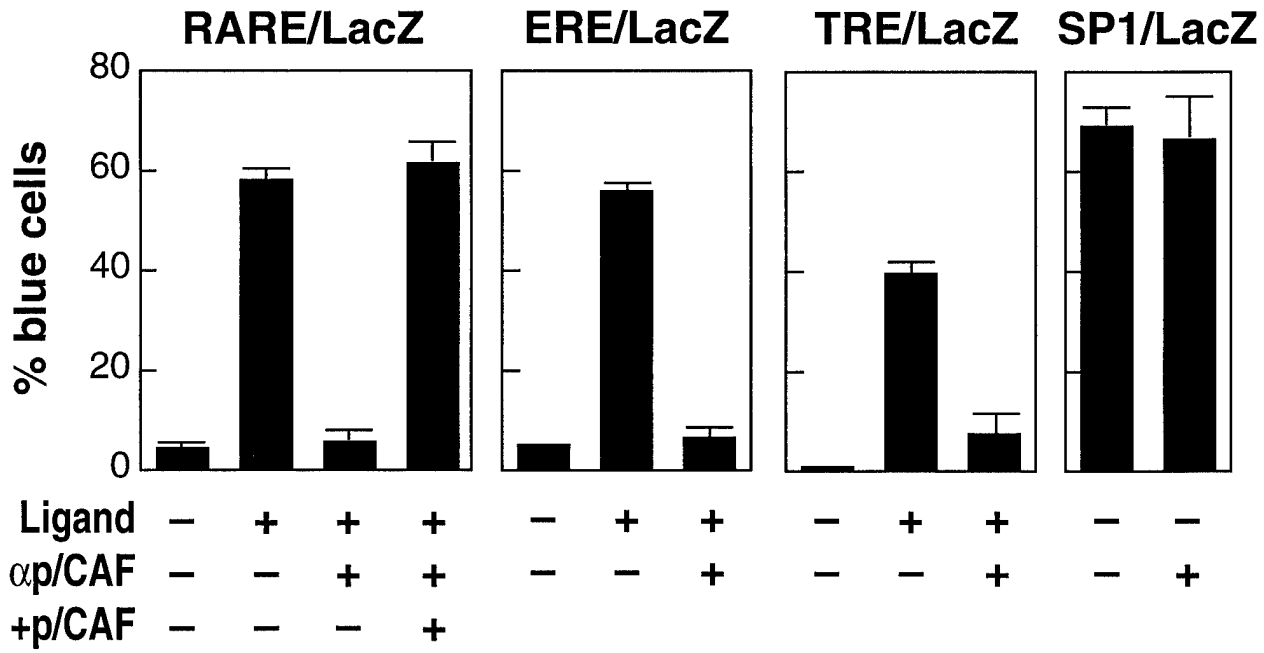

RARE/LacZ
ERE/LacZ
TRE/LacZ
SP1/LacZ
% blue cells
80
60
40
20
0
Ligand – + + + – + + – + + – –
αp/CAF – – + + – – + – – + – +
+p/CAF – – – +

Figure 1.

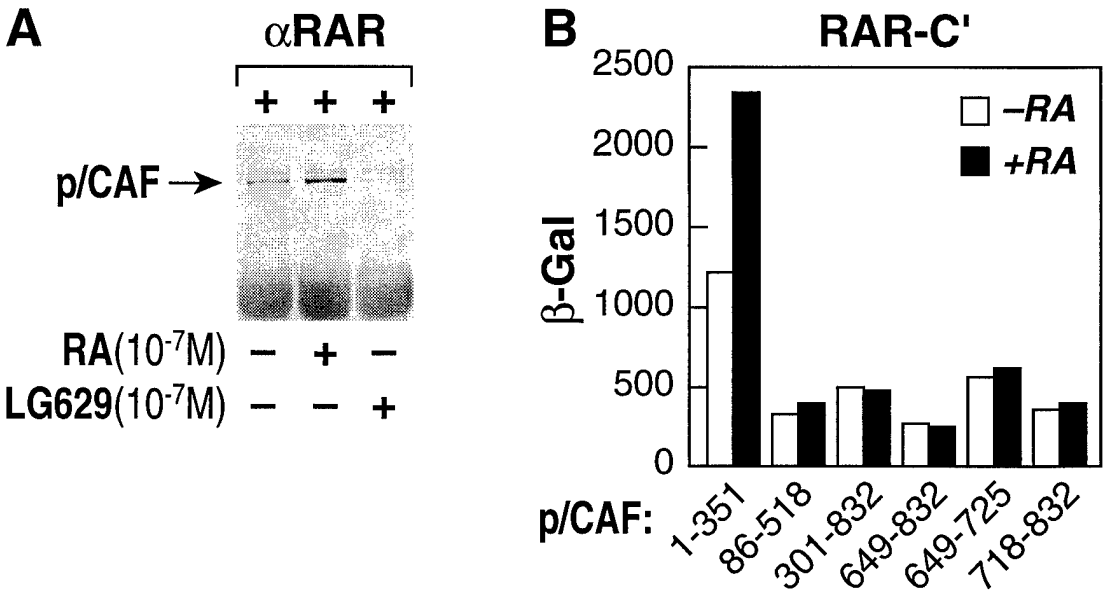

A
αRAR
+ + +
p/CAF →
RA(10-7M) – + –
LG629(10-7M) – – +
B
RAR-C'
2500
2000
1500
1000
500
0
β-Gal
–RA
+RA
p/CAF:
1-351
86-518
301-832
649-832
649-725
718-832

Figure 2.

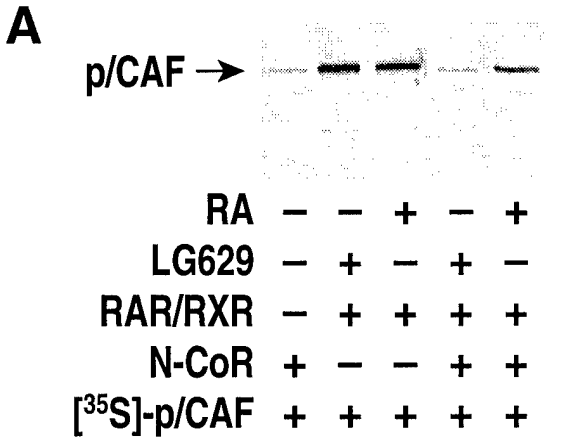

A
p/CAF
RA – – + – +
LG629 – + – + –
RAR/RXR – + + + +
N-CoR + – – + +
[35S]-p/CAF + + + + +
B
p/CAF
TTNPB – – +
LG629 – + –
RARΔAF2/RXR – + +
[35S]-p/CAF + + +

Figure 3.

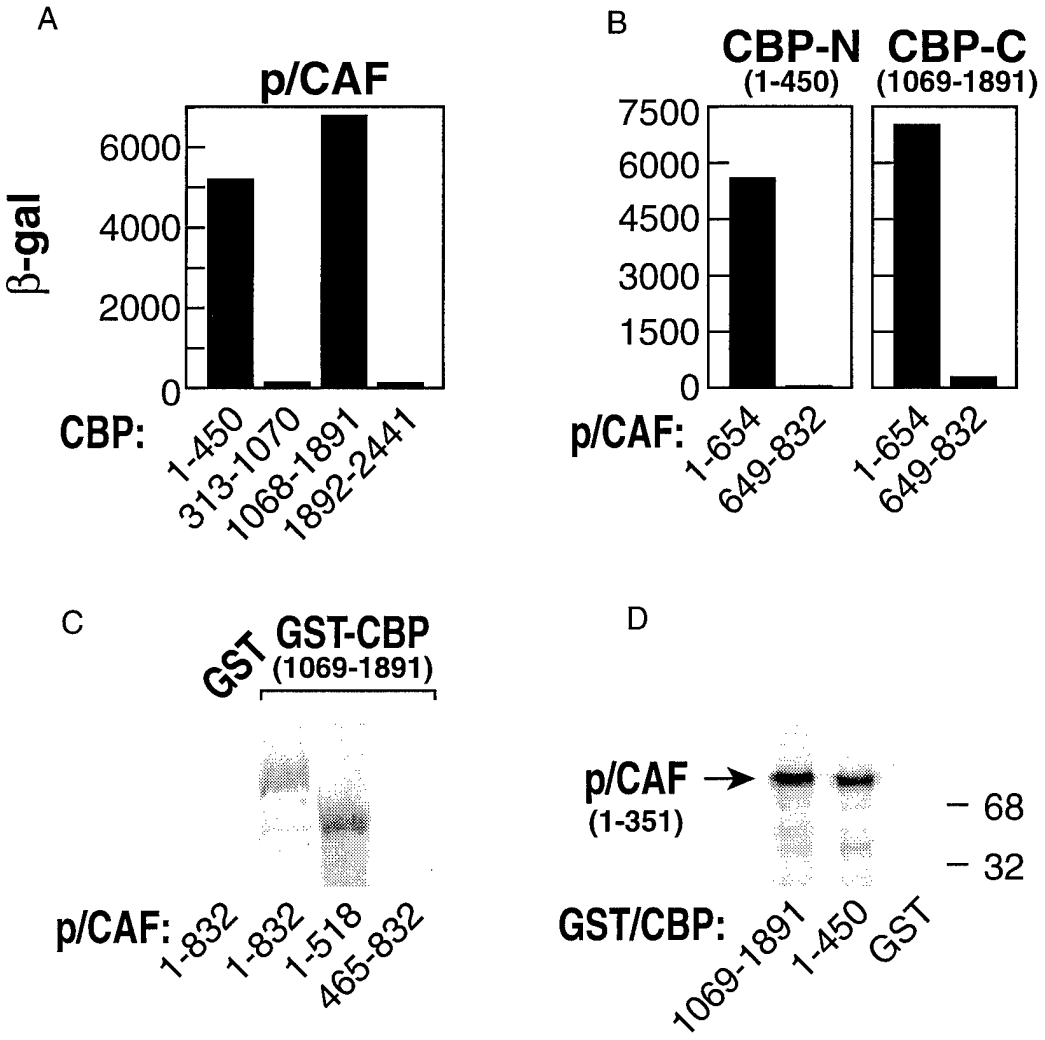
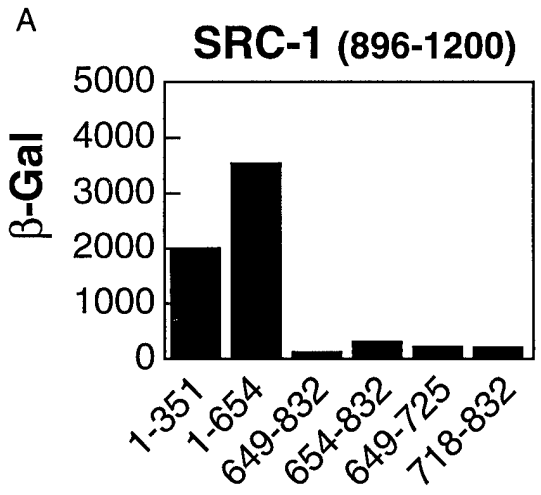
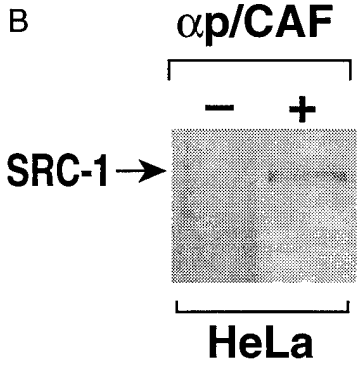
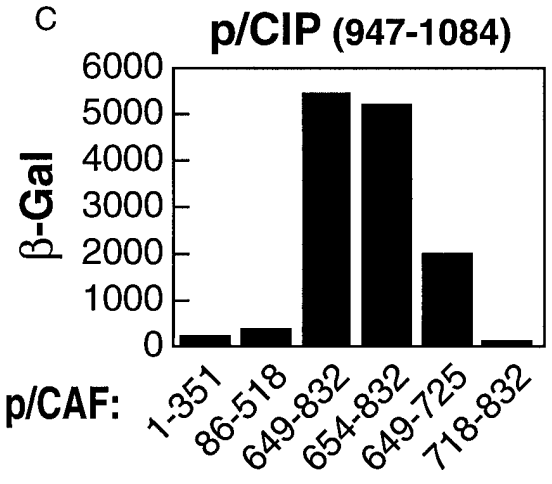

A
p/CAF
β-gal
6000
4000
2000
0
CBP:
1-450
313-1070
1068-1891
1892-2441
B
CBP-N
(1-450)
CBP-C
(1069-1891)
7500
6000
4500
3000
1500
0
p/CAF:
1-654
649-832
1-654
649-832
C
GST
GST-CBP
(1069-1891)
p/CAF:
1-832
1-832
1-518
465-832
D
p/CAF
(1-351)
68
32
GST/CBP:
1069-1891
1-450
GST

Figure 4.


A
SRC-1 (896-1200)
β-Gal
5000
4000
3000
2000
1000
0
1-351
1-654
649-832
654-832
649-725
718-832


B
αp/CAF
−
+
SRC-1
HeLa


C
p/CIP (947-1084)
β-Gal
6000
5000
4000
3000
2000
1000
0
p/CAF:
1-351
86-518
649-832
654-832
649-725
718-832

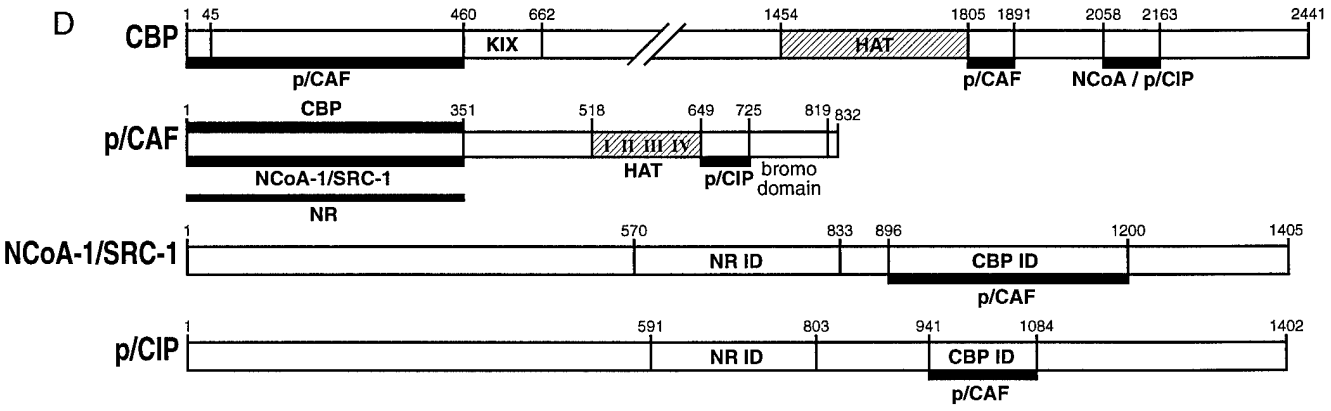

D
CBP
1
45
460
662
1454
1805
1891
2058
2163
2441
KIX
HAT
p/CAF
p/CAF
NCoA / p/CIP
p/CAF
CBP
1
351
518
649
725
819
832
I II III IV
HAT
p/CIP
bromo domain
NCoA-1/SRC-1
NR
NCoA-1/SRC-1
1
570
833
896
1200
1405
NR ID
CBP ID
p/CAF
p/CIP
1
591
803
941
1084
1402
NR ID
CBP ID
p/CAF

Figure 5

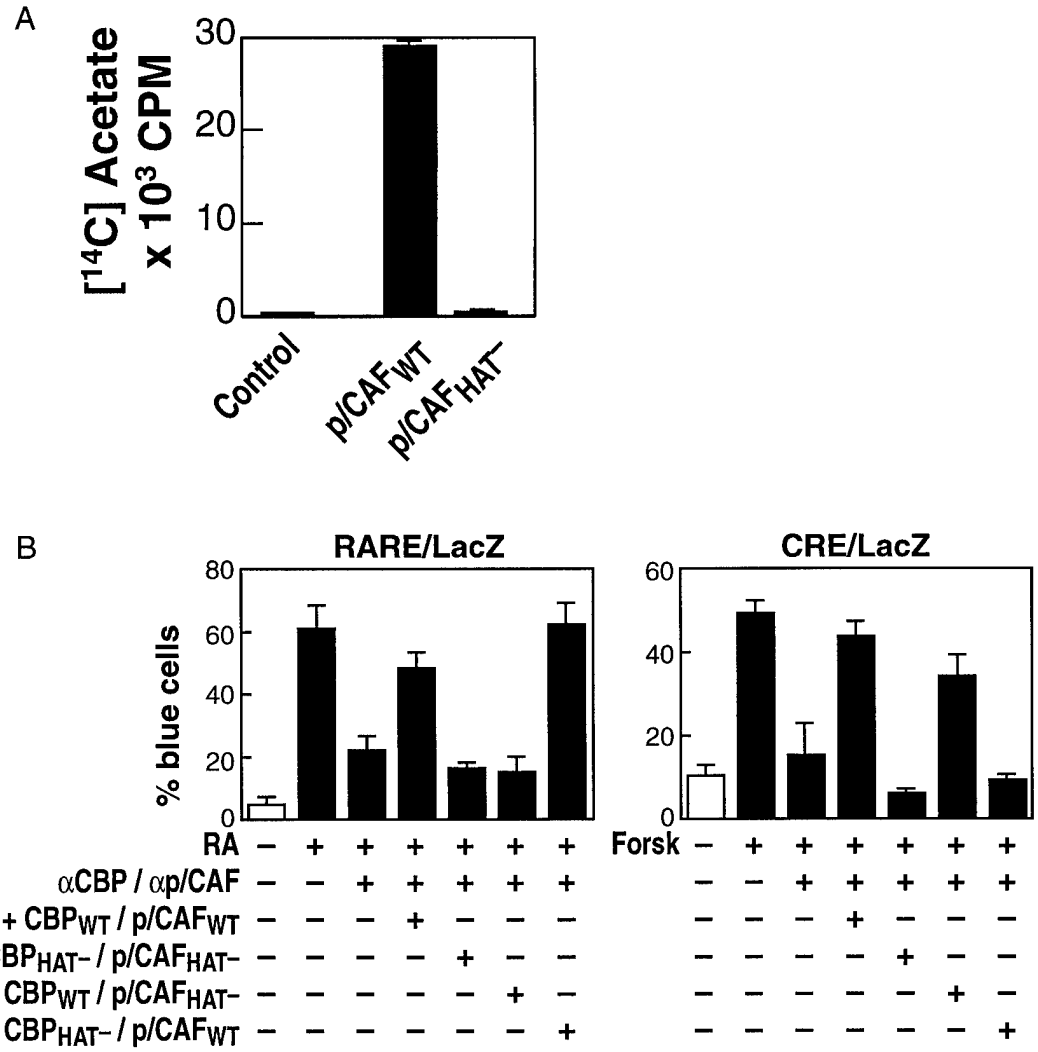

A
[14C] Acetate
x 10³ CPM
30
20
10
0
Control
p/CAF WT
p/CAF HAT–
B
RARE/LacZ
CRE/LacZ
% blue cells
80
60
40
20
0
RA
Forsk
αCBP / αp/CAF
+ CBP WT / p/CAF WT
+ CBP HAT– / p/CAF HAT–
+ CBP WT / p/CAF HAT–
+ CBP HAT– / p/CAF WT

Figure 6.

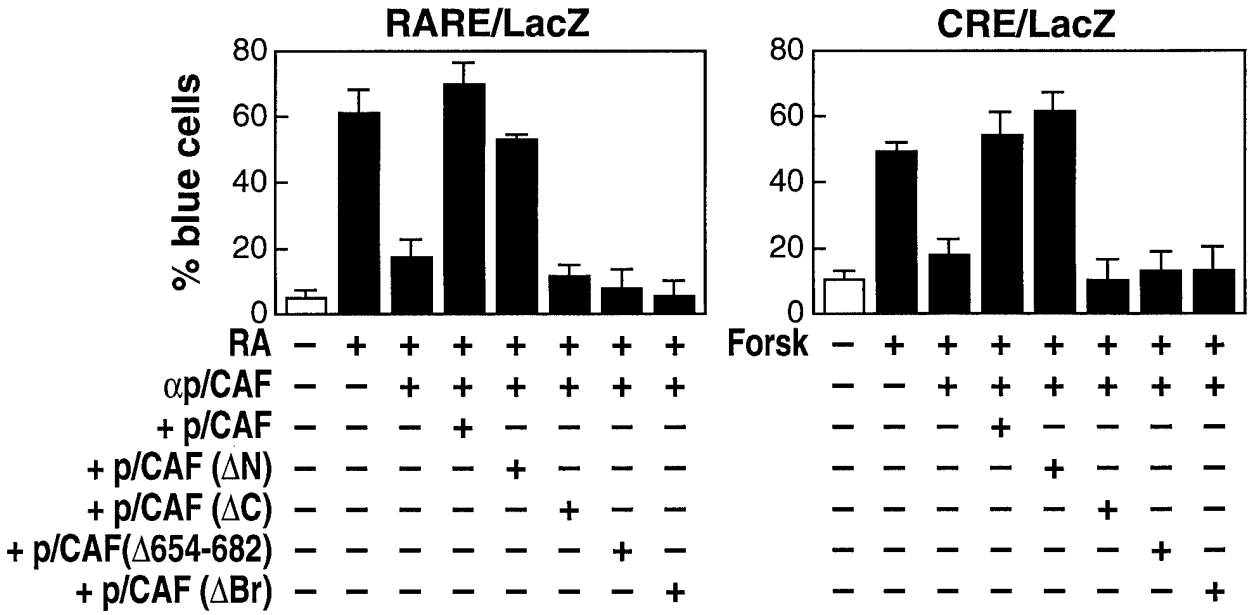

RARE/LacZ
CRE/LacZ
% blue cells
80
60
40
20
0
RA – + + + + + + +
αp/CAF – – + + + + + +
+ p/CAF – – – + – – – –
+ p/CAF (ΔN) – – – – + – – –
+ p/CAF (ΔC) – – – – – + – –
+ p/CAF(Δ654-682) – – – – – – + –
+ p/CAF (ΔBr) – – – – – – – +
Forsk – + + + + + + +
– – + + + + + +
– – – + – – – –
– – – – + – – –
– – – – – + – –
– – – – – – + –
– – – – – – – +

Figure 7.

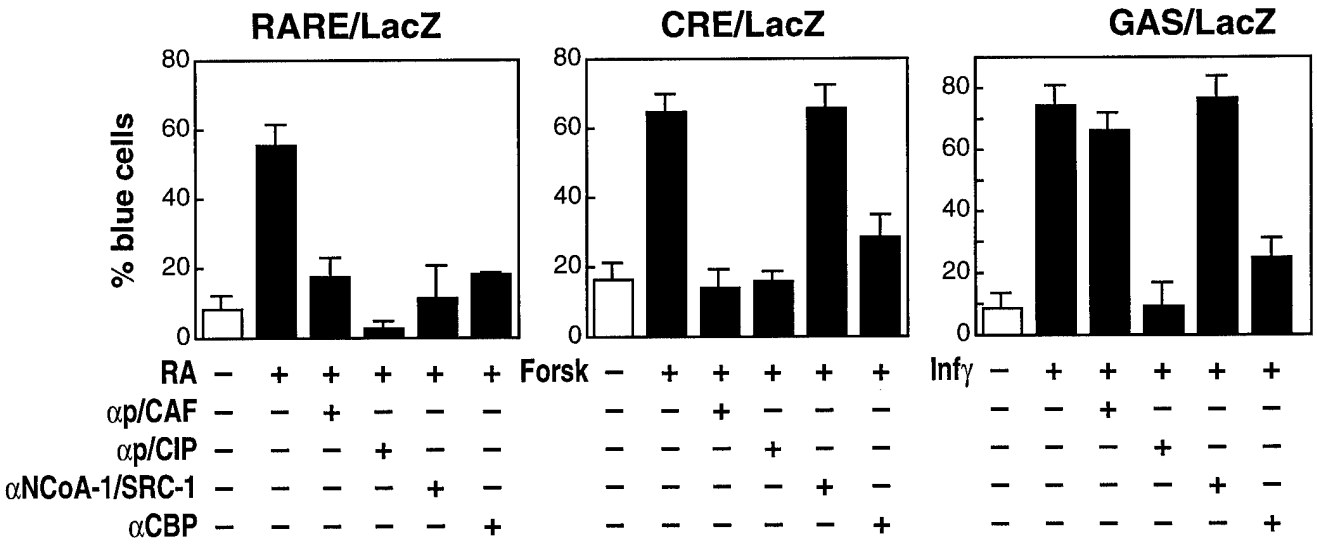

RARE/LacZ
CRE/LacZ
GAS/LacZ
% blue cells
80
60
40
20
0
RA – + + + + +
αp/CAF – – + – – –
αp/CIP – – – + – –
αNCoA-1/SRC-1 – – – – + –
αCBP – – – – – +
Forsk – + + + + +
– – + – – –
– – – + – –
– – – – + –
– – – – – +
Infγ – + + + + +
– – + – – –
– – – + – –
– – – – + –
– – – – – +

Figure 8.